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R222Q Nav1.5 Mutation Associated with a New SCN5A-Related Cardiac Arrhythmia
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Using a candidate-gene approach, we detected a variant of SCN5A, encoding the cardiac Na^+ channel Nav1.5, by screening a family with cardiac arrhythmia resulting in frequent premature ventricular contractions (PVCs) and non-sustained ventricular tachycardia. Arrhythmia mechanism involved ectopic foci originating from the His-Purkinje system. The same mutation, leading to the R222Q substitution, was present in two additional unrelated families with the same associated cardiac phenotype. Exercise or hydroquinidine dramatically decreased the number of PVCs. To evaluate the functional incidence of this substitution, whole-cell patch-clamp experiments were performed on transfected COS-7 cells. The activation and inactivation curves were negatively shifted in the presence of the mutation ($V_{1/2act}$, WT: -30.6 ± 2.1 mV, n=9; heterozygous: -37.2 ± 1.6 mV, n=9; $p<0.05$; $V_{1/2inact}$, WT: -79.6 ± 0.7 mV, n=10; heterozygous: -82.2 ± 1 mV, n=9; $p<0.05$) whereas the current density was unchanged. The use of depolarizing-voltage ramp confirmed the increase and negative shift of the TTX-sensitive window current (potential of g_{max}, WT: -42.8 ± 0.5 mV, n=12; homozygous: -58.6 ± 1.1 mV, n=, $p<0.001$). WT and R222Q peak I_{Na} were similarly half-reduced by 30 µM quinidine ($p<0.001$ vs control) as well as the window current (WT: from -1.87 ± 0.42 to -0.74 ± 0.16 pA/pF in quinidine, n=7; homozygous: -2.54 ± 0.41 to -1.17 ± 0.12 pA/pF in quinidine, n=6; $p<0.001$ vs. control).
We carried out computer simulations in single-cell models of human Purkinje fibers and ventricle action potentials (AP). In heterozygous conditions, incomplete repolarization occurred in Purkinje cells only. We also built a multicellular model incorporating both cell models. In the heterozygous conditions, incomplete repolarization in the Purkinje fibers triggered premature APs propagating into the ventricle. This was normalized at higher pacing frequency or when quinidine was 'added'.
From all these studies, the premature ventricular contractions are explained by the appearance of electrical abnormalities rather in Purkinje fibers than in ventricular cardiomyocytes.

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Role of the Cytoplasmic N-Terminal Domain of the Cardiac Sodium Channel Alpha-Subunit
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Heterozygous loss-of-function mutations in the *SCN5A* gene encoding the cardiac Na^+ channel $Na_v1.5$ cause various hereditary arrhythmias including Brugada syndrome (BrS), a disease characterized by ST-segment elevation in the ECG right precordial leads, and an increased risk of sudden cardiac death. Here, we aimed to study the role of the $Na_v1.5$ evolutionarily conserved N-terminus of which the function remains unknown. We characterized two mutations within the cytoplasmic N-terminus of $Na_v1.5$, R104W and R121W identified in BrS patients and a construct where this region was deleted. In HEK cells, R104W, R121W and Nav1.5-ΔNter abolished I_{Na}. Immunostaining of Nav1.5 transfected in neonatal rat cardiomyocytes showed retention of R104W and R121W in cytoplasmic compartments, as opposed to the WT channels. Moreover, Western blot analysis revealed that mutants were mostly degraded and that the ubiquitin proteasome inhibitor, MG132, prevented this degradation. Coexpression of WT with either mutants, mimicking the heterozygous state of BrS patients, led to a marked I_{Na} density reduction (80%) and a 8 mV positive shift of the $V_{0.5}$ activation, compared to WT alone, demonstrating a dominant-negative effect. Interestingly, when $Na_v1.5$-ΔNter was coexpressed with WT, no dominant-negative effect was observed. We demonstrated that (i) the two N-terminal mutation abolish I_{Na} through retention of the mutant channels and their subsequent degradation by the proteasome, (ii) the two mutant channels exert a specific dominant-negative effect on WT channels by producing a default in WT channel trafficking to the membrane, (iii) these mutations have more drastic effects on I_{Na} than deletion of this region. Altogether, our results suggest that the N-terminal domain of Nav1.5 plays an important role in the traffic of Nav1.5 subunits to the plasma membrane, the regulation of their expression and their activation.

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The Role of Channel PKA/PKC Sites in Metabolic Regulation of the Cardiac Na^+ Channel
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Background: Intracellular NADH downregulates cardiac Na^+ current (I_{Na}) acutely to a magnitude seen in Brugada Syndrome by activating protein kinase C (PKC). The decrease in I_{Na} can be ameliorated by protein kinase A (PKA) activators. PKC and PKA down- and upregulates I_{Na}, respectively. Here, we studied the roles of known channel phosphorylation sites.
Methods: HEK293 cells transfected with human cardiac Na^+ channel wild type (WT), S1503A, S1503D, S525/528A, and RRR533-535AAA, were utilized for whole-cell patch clamp recording. Peak I_{Na} was measured at −30 mV with holding potential at −100 mV. All data were compared with WT.
Results: The peak I_{Na} of all mutants was similar to that of the WT channel. Compared to the WT $Na_v1.5$, the peak I_{Na} obtained with application of 100 µM NADH decreased to $41 \pm 5\%$ ($P<0.01$). Application of NADH failed to reduce I_{Na} of S1503A ($81 \pm 6\%$) but not of S1503D ($42 \pm 2\%$, $P<0.01$). PMA, a PKC activator, decreased I_{Na} of WT and S1503D ($47 \pm 5\%$ and $37 \pm 6\%$, respectively, $P<0.01$), but not that of S1503A ($87 \pm 11\%$, $P>0.05$). The NADH-induced decrease in I_{Na} could be ameliorated with the PKA activator, forskolin in S525/528A ($92 \pm 10\%$) but not RRR533-535AAA ($47 \pm 5\%$, $P<0.01$). The latter mutation also prevented NAD^+ mediated current recovery. **Conclusions**: The relatively retained current with the 1503 mutations alone and the lack of effect on I_{Na} by PKC activation of 1503A but not 1503D suggests that phosphorylation at this site is necessary but not sufficient to explain the NADH-dependent reduction in I_{Na}. The PKA-dependent rescue of current seems to be dependent on phosphorylation of the channel at the RRR533-535 sites.

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Functional Analysis of Stably Expressed Human Nav1.9
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The tetrodotoxin (TTX)-resistant voltage gated sodium channels, $Na_V1.8$ and $Na_V1.9$, are important for neuronal pain pathways. $Na_V1.9$ (a.k.a. NaN) is preferentially expressed in nociceptive neurons of dorsal root ganglia. The role of $Na_V1.9$ in inflammatory and neuropathic pain along with a restricted cellular localization makes it an attractive target for novel analgesics. Most of our knowledge about $Na_V1.9$ function comes from studies of rodent sensory neurons using internal solutions and recording protocols to differentiate $Na_V1.8$ and $Na_V1.9$ currents, as well as recent studies using neurons isolated from $Na_V1.8$-null mice. To further elucidate the functional properties of $Na_V1.9$, we developed a stable cell line expressing full length human $Na_V1.9$. ND7/23 cells were stably transfected using a novel transposon system in combination with human β1 and β2 sodium channel accessory subunits. Whole-cell currents were recorded from a holding potential of −120 mV and elicited with 20 ms pulses from −80 to +50 mV in the continuous presence of 200 nM TTX to block an endogenous TTX-sensitive sodium current. We recorded slow-activating, persistent sodium currents in $Na_V1.9$ expressing cells following 24 h incubation at 28°C (to boost cell surface expression). The whole-cell current peaked at −30 mV (15.2 ± 1.8 pA/pF, n=26) and exhibited a voltage-dependence of activation with V½ of −50.5 ± 1 mV and slope factor (k) of 7.2 ± 0.3 (n=26). Activation kinetics was much slower than that observed for neuronal, TTX-sensitive Na_V channels; time to peak current (at −30 mV) was 12.6 ± 0.7 ms, n=26. Preliminary single channel recording demonstrated frequent late re-openings and a single channel conductance of ~16 pS. These results demonstrate the biophysical properties of stably expressed human $Na_V1.9$ channel and provide a cell based platform for the analysis of potential $Na_V1.9$ therapeutic agents.

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Neuronal Sodium Channels Contribute Significantly to the Cardiac Repolarization during Ischemia
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Introduction: Infarcts are responsible for cardiac arrhythmias and transient ischemic episodes. It has been demonstrated that the neuronal sodium channels (nNa_vs) are overexpressed around an ischemic area. Ischemias induce a depolarization of the membrane potential and it's known that nNa_vs channels activate at more depolarized potentials than the cardiac sodium channels (Nav1.5). We hypothesised that this overexpression of nNavs channels may act as a safety factor for cardiac conduction during ischemia. However, the nNavs have a larger late component than Nav1.5 therefore prolonging the action potential duration (APD) and creating an arrythmogenic substrate (AS). The aim of this

study is to estimate the contribution of the nNavs in the late sodium current (I_{NaL}) in ischemic condition in order to determine if an overexpression of the nNa_vs can modify the APD and create an AS.
Methods: Freshly isolated cardiomyocytes were placed under ischemic conditions for 45 minutes. I_{NaL} currents were recorded with the patch clamp technique in whole cell configuration. Tetrodotoxin (a specific nNa_vs blocker) and MTSEA (a specific $Na_v1.5$ blocker) were used to differentiate the two sodium channels isoforms.
Results: In normal condition, nNa_vs account for 11% of peak current. I_{NaL} represents 0.3% of the peak current at a potential of −10 mV. Contribution of nNa_vs (TTX sensitive) to I_{NaL} was 36% ± 5%. Ischemia decreases the maximal current density from −73.3 nA/pF to −53.4 nA/pF. Surprisingly, nNa_vs contribution was not modified (10% of the peak current). However, ischemia increases I_{NaL} from 0.3% to 1.6% compared to the peak current.
Conclusion: Ischemia increases by 5.3 times I_{NaL} that can play a critical role in the duration of the action potential and facilitates the outcome of arrhythmias.

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Analysis of Voltage-Gated Sodium Channel Membrane Dynamics in Hippocampal Neurons via a Fluorescent Protein and Biotin Tagged Nav1.6 Channel
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Voltage-gated sodium channels (Nav) are densely accumulated at the axon initial segment (AIS) of neurons where they are responsible for action potential initiation. The dense clustering of channels at the AIS involves ankyrinG binding, however the details of trafficking these channels to the AIS remains elusive. Furthermore, it is unclear what percentage of AIS channels is actually conducting. Since the large sodium channel cDNAs are difficult to manipulate and suffer from rearrangements in E. coli, the most elegant trafficking work to date has utilized chimeric proteins containing the sodium channel ankyrin-binding motif fused to other membrane proteins. To fully address trafficking in real time, an appropriately tagged full-length and functional channel is required. Therefore, we developed a Nav1.6 tagged with either GFP or Dendra2 fluorescent-proteins on the C-terminus and an extracellular biotin-acceptor-domain (BAD). The BAD allows for visualization and single molecule tracking of quantum-dot-bound sodium channels on the neuronal surface. This modified Nav1.6 demonstrated wild-type activity when expressed in hippocampal neurons. The tagged channel efficiently trafficked to the cell surface and was localized at the AIS as indicated by both confocal and TIRF microscopy. Alexafluor 594-conjugated-streptavidin binding indicated the surface-density of channels at the AIS was approximately 60 times greater than on the soma, comparable to endogenous Nav1.6 channels. Fluorescence recovery after photobleaching (FRAP) and single particle tracking showed that channels at the AIS had recovery time constants of greater than 2 hours and were confined to 60nm +/- 20nm compartments. In summary, we constructed a sodium channel with fluorescent protein and extracellular biotin reporters that has both wild-type trafficking and biophysical properties. This construct will permit the examination of sodium channel turnover, trafficking, diffusion and location-dependent function in neuronal cells.

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NaV1.7 Splice Variant from Human Heart Compared with Neuronal hNaV1.7
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Perception of noxious stimuli can be profoundly affected by mutations in the gene SCN9A which encodes the α-subunit of the voltage-gated sodium channel NaV1.7. Mutantions of this channel are associated with chronic pain or complete absence of pain. Primary erythermalgia and paroxysmal extreme pain disorder are syndromes associated with attacks of severe pain resulting from mutations that enhance NaV1.7 channel activity. Non-sense mutations in SCN9A lead to complete loss of NaV1.7 function. Loss of NaV1.7 function produces complete insensitivity to pain and anosmia, but little other changes in functions or behaviors. The pain-specific nature of the mutant NaV1.7 phenotypes is in keeping with the notion that this channel is expressed primarily in dorsal root ganglia and, to a lesser extent in the sympathetic ganglia. These premises make NaV1.7 an ideal target for the development of novel non-addictive analgesics. However, expression of NaV1.7 has been detected in the heart, with 5- to 10-fold higher levels in human cardiac Purkinje fibres versus the right atrium and ventricle and bradycardia and cardiac asystole have been reported in patients with paroxysmal extreme pain disorder. While these events have usually been ascribed to autonomic effects of the NaV1.7 mutations, a more direct effect of altered NaV1.7 in the heart cannot be ruled out. We have set out to clone and characterize the specific NaV1.7 subtype expressed in the human heart. In the present study we cloned and characterized a NaV1.7 subtype predominantly expressed in the human heart. This novel splice variant, missing one exon, may impact drug safety for this emerging family of analgesics. Its biophysical and pharmacological properties have been studied and will be discussed in comparison to the neuronal variant.

2690-Pos Board B460
Biophysical and Pharmacological Characterisation of Native Human $Na_V1.8$ Channels from Isolated Dorsal Root Ganglia (DRG)
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$Na_V1.8$ is a tetrodotoxin resistant (TTX-r) sodium channel expressed in sensory neurones that has a depolarised activation threshold, slow inactivation kinetics and a recovers rapidly from inactivation compared to tetrodotoxin sensitive (TTX-s) channels (Cummins & Waxman 1997; Akopian *et al.,* 1996). These biophysical properties mean that $Na_V1.8$ contributes to both electrogenesis and the maintenance of repetitive firing of action potentials (Blair & Bean 2002; Renganathan *et al.,* 2001; Waxman *et al.,* 2001). The expression and biophysical properties of $Na_V1.8$ can be modulated by ongoing nociceptive input and findings in the literature strongly support a key role for $Na_V1.8$ in pain signalling (England *et al.,* 1996; Roza *et al.,* 2003; Kerr *et al.,* 2001; Coward *et al.,* 2000; Akopian *et al.,* 1999). The selective $Na_V1.8$ antagonist A803467 has provided further evidence for a role of $Na_V1.8$ in nociceptive sensory input (Jarvis *et al.,* 2007) however characterisation and pharmacology of native $Na_V1.8$ currents has been shown using cells isolated from non human species. We have characterised $Na_V1.8$ currents recorded from TTX-r channels in human DRG and shown that they can be inhibited by a selective modulator of TTX-r current, A803467. Isolated currents had a $V_{1/2}$ inactivation of −35 mV and a $V_{1/2}$ of activation of −10 mV. Action potentials evoked by increasing current injections were inhibited by TTX and by the selective $Na_V1.8$ modulator A803467 which abolished repetitive firing. Furthermore, we have compared the biophysical properties of the native channel with those of recombinantly expressed human $Na_V1.8$ channel. This work not only provides a means by which we can assess the biophysics and pharmacological modulation of native human $Na_V1.8$ currents, but will also help us to understand the role of the channel in human pain signalling.

2691-Pos Board B461
Input Signal Patterns Dominate a Plasticity of Spike-Onset Location at Cortical Pyramid Neurons through Local VGSCs
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Synaptic signals drive the neurons to fire sequential spikes as digital codes. Short-term pulses initiate a spike at the axonal hillock, and physiological signals may initiate digital spikes at the soma. The regulation, mechanism and impact for spike-onset relocation between subcellular compartments remain unknown, which we investigated by simultaneously recording the soma and axon of pyramidal neurons in cortical slices. By analyzing the abilities of firing spikes, the time phases of spike-onset and the relocations of spike-initiation at these two compartments, we have found that long-time steady depolarization induces sequential spikes at the soma, but fluctuated one induces spikes at the axon. The soma in response to long-time pulses shows low thresholds and short refractory periods, or vice versa. Compared with axonal voltage-gated sodium channels (VGSC), somatic VGSCs in response to a pre-depolarization appear less inactivated and easily reactivated. Based on these VGSC features, the location of spike-onset simulated in *NEURON* model is consistent with the experiments. The patterns of input signals dominate spike initiations at the axon or soma of cortical pyramidal neurons through influencing local VGSC function. The plasticity of spike-onset location allows the neurons to program the brain codes economically. [This study is supported by the National Award for Outstanding Young Scientist (30325021), National Basic Research Program (2011CB504405) and Natural Science Foundation China (30870517, 30990261 and 81171033) to JHW].

2692-Pos Board B462
A Markov Model for the Human Cardiac Sodium Channel
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Voltage-gated sodium channels play an important role in the function of the human heart. Different voltage-clamp protocols were employed to determine kinetic and steady state voltage dependences that characterize channel gating. They include activation, deactivation, inactivation, and recovery from inactivation kinetics, current-voltage relationships, steady-state inactivation, and voltage dependence of normalized channel conductance (G/G_{max}). Several attempts were made to develop comprehensive mathematical model for sodium channel, however, most of them have noticeable limitations. We developed